Flow Cytometry Protocol:

* Day Before:

Start overnight cultures of your strains to be tested in 5-10ml SD Complete media. (Do not use YPD. YPD has fluorescence background which would cause problems for FACS testing.)

* Day Of:

Flow Procedures:

1. Dilute overnight cultures 1:100 in the 96 well shaker plate. (Shaker plates can be found in the Lim Lab in the back glass cabinet where large glass bottles can also be found.)
2. Allow cells to enter growth stage by putting on plate shaker at 30°C going 1000rpm (found in Flow room or the 30°C room in the other hall)for 2-3 hours after dilution.
3. Induce with alpha factor. The stock alpha factor in the freezer is 3mM and we use it at the final concentrations of: 0, 1nM, 10nM, 100nM, and 1uM.
4. Allow introduction to proceed on plate shaker for 90 min to 2 hrs, no longer.
5. Transfer 250ul of each culture to the 96 well flow cytometry v-bottom plate. (found in flow room on shelf) Arrest cells with 10ul of cycloheximide. (stops translations of proteins; stops growth)
6. Run on flow cytometer.
7. Analyze Data.

Things to check for Flow:

1. Check that machine is on standby and not run. (Should be on standby when not in use)
2. Make sure that a clean plate has been ran by the person before you. Otherwise, run a clean plate. (A1-A4 bleach, B1-B4 water)
3. Check fluid buffer box and storage tanks. Discard waste fluid in sink 2 doors down (remember to add bleach to tank when done) and replace buffer box if needed.
4. Make sure you have a positive and negative control so that you can set the parameters.
5. Open up FACSDiva. Sign into iGEM account.
6. Create new FACS experiment. Highlight needed wells and use the blue button to create wells for use. Create specimens among the wells and rename them.
7. Open up inspector to check parameters.

8. Run well. (Do not click 'Run Plate'.)
9. Once finished, export data onto a USB.
10. Run a clean plate.

*** Note: NEVER TURN THE WIFI ON! THE COMPUTER WILL CRASH!**

Voltage Parameters:

FSC: 250
SSC: 280
FITC: 550

Sample Loading Parameters:

Sample Volume: 200
Mixing Volume: 150
Flow Speed: 0.5ul/sec when adjusting, otherwise 1ul/secd
Mixing quantity: 3

Using Flowjo and Matlab to Analyze Data

Flowjo:

1. Export Data from USB and transfer to iGEM2014 folder.
2. Open Flowjo and drag data over to box.
3. Set an appropriate gate.
4. Go to data, select all data and press 'Σ'. Select median, mean, and count.
5. Press the refresh button. Additional numerical data should be present.

Matlab

1. Open up a new script. and comment the title of the script. (Use '%' for comments.)
2. Enter the alpha factor info that will serve as the x-axis. (Put in log form.)
3. Enter the yGEM data. This will serve as the y-axis.
4. Type your figure info and press the "go".

Alpha Factor Dilutions:

1x	100x
0 nm	0 nm
0.5 nm	50 nm
1 nm	100 nm
10 nm	1000 nm
100 nm	10000 nm
1000 nm	100000 nm
3000 nm	300000 nm

5 µl 10 mM stock + 495 µl H2O = 100,000 nm
50 µl 100,000 nm + 450 µl H2O = 10,000 nm
50 µl 10,000 nm + 450 µl H2O = 1,000 nm
50 µl 1000 nm + 450 µl H2O = 100 nm
200 µl 100 nm + 200 µl H20 + 50 nm

Time Course

0, 1.5, 3, 5 hours

Dox concentrations

0, 0.03, 0.06, 0.09, .6, 6

Dox Dilutions:

Stock: 50 mg/mL

1x	**100x**	Prep
60µg/mL	A: 6mg/mL	60µL of Stock in µL 440µL of water
30µg/mL	B: 3mg/mL	30µL of Stock in 470µL of waterb
9µg/mL	C: 900µg/mL	75 µL of A in 425µL of water
6µg/mL	D: 600µg/mL	50 µL of A in 450 µL of water
3µg/mL	E: 300µg/mL	25 µL of A in 475 µL of water
0.9µg/mL	F: 90µg/mL	50 µL of C in 450µL of water
0.6µg/mL	G: 60µg/mL	50 µL of D in 450µL of water
0.3µg/mL	H: 30µg/mL	50 µL of E in 450µL of water
0.09µg/mL	I: 9µg/mL	50 µL of F in 450µL of water
0.06µg/mL	J: 6µg/mL	50 µL of G in 450µL of water
0.03µg/mL	K: 3µg/mL	50 µL of H in 450µL of water

Dilutions to same OD for mixing strains

Actual OD / Target OD = Dilution Factor

x/0.015 =DF
y/0.015 = DF

µl / Dilution Factor = µl to be added

bb Otherwise, 1:200 dilution.

1mL SD + 5µl culture